IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	:	10/705,432	Confirmation No. 4884
Applicant	:	Auerbach et al.	
Filed	:	10 November 2003	
TC/A.U.	:	1632	
Examiner	:	Montanari, David A.	
Docket No.	:	784	
Customer No.:		26693	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR § 1.132

Sir:

I, David Frendewey, Ph.D., declare as follows:

1. I am a co-inventor of the invention described and claimed in the above-identified patent application. I presently hold the position of Director, Targeted ES Cell Production at Regeneron Pharmaceuticals, Inc., having its principal place of business at 777 Old Saw Mill River Road, Tarrytown, New York 10591.

2. I am aware that the instant application claims our method of targeting mouse ES cells with a targeting vector having a ubiquitin promoter, a drug resistance gene under control of the ubiquitin promoter, and homology arms directing the targeting vector to a pre-selected chromosomal location.

3. The above-referenced specification describes the results of experiments in which targeting vectors having different promoters were constructed and tested for gene targeting to different mouse genes. The vectors contained homology arms that targeted the vector to a desired pre-selected gene locus, a drug resistance gene, and a promoter controlling expression of the drug resistance gene. Table 2 as filed provided data for targeting vectors

containing PGK or hUBC promoters, although a third vector containing the SV40 promoter was also tested at the same time. Table 2 provided results for the number of drug-resistance colonies generated with PGKp and hUBCp vectors, the number of clones screened for correct targeting, and the number of clones identified as correctly targeted.

4. Attached Table A is a clearer presentation of the data previously shown in Table 2, and additionally includes the number of colonies obtained with a third vector (SV40p). In addition, Table A includes the common name, symbol, and Entrez Gene ID number of each gene targeted.

5. Following the methods described in the above-referenced specification at paragraphs [0027]-[0033], [0041]-[0044] and Table 2, the targeting vector was introduced into the ES cells by electroporation, and the ES cells were plated in a medium containing G418. After incubation, G418-resistant colonies developed. A randomly selected subset of clones was screened in order to identify ES cell clones containing genes correctly targeted by the targeting vector. As shown at the last column of Table A or column 8 of Table 2, use of a ubiquitin promoter in a targeting vector averaged almost a 4-fold increase in targeting frequency relative to the PGK promoter.

6. To the best of my knowledge, we were the first to discover that the use of the ubiquitin promoter in a targeting vector could increase targeting frequency. This is a significant discovery in that it dramatically reduces the amount of work needed for screening surviving clones in order to identify correctly targeted cells. The effect seen with the ubiquitin promoter on improved targeting frequency does not appear to be the result of improved promoter strength. In fact, an examination of the expression of the neomycin resistance gene targeted to the F-box protein 25 locus (as measured by quantitative reverse transcription PCR) indicated approximately equal expression from the PGK and ubiquitin promoters (results not shown). Yet the targeting frequency into the same locus was 6.5-fold higher with the ubiquitin promoter (last column, Table A).

7. The results also show the use of the ubiquitin promoter in a targeting vector allows genes to be targeted that were difficult to target with conventional vectors. As seen in Table A, no correctly-targeted clones were obtained when nebulin or IL-1 receptor-like 1

genes were targeted with a PGKp vector, but 1 or 6, respectively, were obtained when the hUBCp vector was used.

8. The results show that the use of the ubiquitin promoter in a targeting vector is an improvement over the prior art in that it reduces the number of colonies needed to be screened in order to identify a desired correctly targeted clone. Further, the results show that use of the ubiquitin promoter in a targeting vector allows targeting of genes that we were not able to target with a conventional targeting vector.

9. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

25 July 2006
Date

David Frendewey

David Frendewey, Ph.D.
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591
(914) 344-7400

TABLE A

Gene (Name/Symbol/Entrez Gene ID)	Number of Clones Obtained			Correctly Targeted/Screened		Fold Increase
	PGKp	hUBCp	SV40p	PGKp	hUBCp	
N (Nebulin/Neb/17996)	32	96	2	0/32	1/96 (1.0%)	n.d.
F (F-box protein 25/FBXO25/26260)	42	291	1	1/40 (2.5%)	47/288 (16.3%)	6.5
P (parathyroid hormone receptor 2/PTHR2/213527)	97	200	n.d.	1/96 (1%)	7/192 (3.6%)	3.6
T (cyclic AMP-regulated phosphoprotein, 21/Arpp21/74100)	270	1804	15	2/144 (1.38%)	19/576 (3.30%)	2.4
1R7 (interleukin 18 receptor accessory protein/Il18rap/16174)	224	960	n.d.	1/192 (0.5%)	5/288 (1.74%)	3.5
S (interleukin 1 receptor-like 1/ Il1rl1/17082)	411	2444	n.d.	0/288	6/288 (2.08%)	n.d.
20 (interleukin 20/Il20/58181)	477	1436	n.d.	3/288 (1.04%)	6/288 (2.08%)	2.0
E (Eph receptor A6/Epha6/13840)	591	2370	n.d.	4/288 (1.39%)	19/288 (6.60%)	4.75
D (discoidin domain receptor family, member 2/Ddr2/18214)	20	313	0	n.d.	0/288	n.d.